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(54) **Title:** NUCLEIC ACID ISOLATION

(57) **Abstract**

A method for isolating nucleic acids from a biological sample by dissociating protein present in the biological sample, adding a sufficient amount of an inorganic or organic salt to precipitate protein, mixing the biological sample containing the salt for a time sufficient to achieve a satisfactory dispersion of the salt through said biological sample to obtain a protein precipitate, separating the precipitated protein from the biological sample, and separating the nucleic acid remaining in the biological sample. The salt is preferably ammonium acetate and room temperature ethanol is preferably used to precipitate and recover the nucleic acid.

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NUCLEIC ACID ISOLATION

Field of the Invention

The present invention relates to a method for
isolating, i.e., separating, nucleic acids from a
biological composition. More particularly, this

5   invention relates to the separation of protein from a
biological sample containing nucleic acids using salt.
The protein is removed prior to the isolation of the
nucleic acids.

Description of the Prior Art

10   The isolation of nucleic acids, i.e., DNA and
RNA, from cellular debris is an important and routine
procedure in the area of biotechnology. Thus, numerous
researchers have attempted to simplify this isolation
procedure. If the nucleic acids are purified from

15   complex mixtures of molecules such as cell lysates,
generally a proteolytic enzyme such as pronase,
proteinase K, chymotrypsin, trypsin, subtilisin,
carboxypeptidase and the like or an ionic detergent
such as sodium dodecyl sulfate (SDS), sarkosyl and the

20   like is used to dissociate or denature most of the
protein which is present. The proteolytic enzymes used
are generally selected to be active against a broad
spectrum of proteins in order to effectively degrade as
much as possible. The protein must be separated or

25   removed from the cell lysate prior to the isolation of
the nucleic acids otherwise the protein copurifies
along with the nucleic acids. In addition, the
protein may interfere with restriction enzymes and with
the separation. A poor spectrophotometer reading may

30   also be obtained. The dissociation of protein from the
nucleic acid as well as the subsequent removal of

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protein is an important step in the isolation of
nucleic acids. After degrading the protein, the
nucleic acid is typically extracted using an organic
solvent.

5   Marmar, J., "A Procedure for the Isolation of
Deoxyribonucleic Acid from Micro-organisms", J. Mol.
Biol., Vol. 3, pp. 208-218 (1961) describes a procedure
to extract high molecular weight DNA from
microorganisms. This procedure is the forerunner of
10   most of today's DNA extraction procedures. Marmar
disrupts cells and removes cell debris and protein by
organic extraction and centrifugation. RNA is degraded
by the action of RNase and is separated from DNA by
differential precipitation of DNA using isopropanol.
15   Degration of DNA is prevented by the presence of
chelating agents and the ionic detergent sodium
dodecyl sulfate.

           At the present time, the most common
technique used to remove the inactivated or digested
20   protein from a biological composition containing
nucleic acids is to use a combination of phenol and
chloroform. It is generally believed that two
different organic solvents are more efficient than one.
The nucleic acid solution is extracted once with
25   phenol, once with a 1:1 mixture of phenol and
chloroform, and once with chloroform. A final
extraction with chloroform or similar compound removes
any lingering traces of phenol from the nucleic acid
preparation. This phenol/chloroform extraction
30   procedure is described in Molecular Cloning by
Maniatis, T. et al., "A Laboratory Manual", published
by Cold Spring Harbor Laboratory, 1982. Maniatis et al
defined "chloroform" as a 24:1 (w/v) mixture of
chloroform and isoamyl alcohol. "Phenol" is described

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as phenol equilibrated with buffer and contains 0.1%
hydroxyquinoline and 0.2% β-mercaptoethanol.

Ether is used to remove any residual traces
of phenol or chloroform from the DNA solution. Ether,
5   of course, is highly volatile and extremely flammable
and should be worked with and stored in an explosion-
proof chemical hood.

After inactivating protein present in a
biological sample which contains nucleic acids,
10   following current techniques, the aqueous layer
containing the nucleic acids in solution is
transferred to another tube and the nucleic acids are
isolated by precipitating the nucleic acids with about
2 volumes of absolute ethanol. This procedure results
15   in about a 67% ethanol final solution. The
precipitated nucleic acids are then recovered by
centrifuging or filtering.

A number of disadvantages are associated with
this use of phenol and chloroform. Both phenol and
20   chloroform are toxic chemicals which require the use of
protective clothing, gloves and fume hoods. Phenol may
be absorbed through the skin in sufficient amounts to
result in death due to its effects upon the central
nervous system as well as damage to the kidneys, liver,
25   pancreas, spleen and lungs. Chronic exposure to low
concentrations of phenol vapor may result in digestive
disturbances, nervous disorders and dermatitis.
Chronic poisoning may be terminal where there has been
extensive damage to the kidney or liver. Dermatitis
30   resulting from contact with phenol or phenol-containing
products is fairly common in industry.

It is also known that phenol oxidation
products can induce DNA damage. This must be taken
into consideration for long term DNA storage. Thus it

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is important to obtain complete removal of phenol
before continuing with the next step in the procedure
which is the isolation of DNA.

Chloroform is a suspected carcinogen and has
5   the ability to cause neoplastic growths. Exposure to
chloroform vapors can result in conjunctivitis and
dilation of the pupils. Prolonged inhalation will
cause paralysis accompanied by cardiac respiratory
failure and finally death. Exposure to chloroform may
10   also cause profound toxemia and damage to the liver,
heart and kidneys.

The phenol and chloroform extraction
procedure is also time-consuming and tedious. The
necessity of using several re-extraction procedures to
15   remove the phenol and the task of carefully removing
the nucleic acid supernatant from the phenol-
chloroform interface makes it a lengthy procedure.

Recognizing the disadvantages associated with
the foregoing procedure, a variety of other techniques
20   have also been disclosed which either modify the
foregoing procedure or use a very different approach.

Gautreau, C. et al, "Comparison of Two
Methods of High-Molecular-Weight DNA Isolation from
Human Leucocytes," Analytical Biochemistry, Vol. 134,
25   pp. 320-324 (1983) describes two methods of DNA
extraction using phenol and chloroform for
deproteinization after protein digestion with
proteinase K and sodium dodecyl sulfate. One method
involves removal of phenol by extensive dialysis with
30   0.01 M sodium tetraborate and 0.01 M EDTA at pH 9.1.
In the second procedure, the DNA was precipitated from
the aqueous layer by the addition of 0.1 volume of 20%
sodium acetate to the aqueous layer followed by the
addition of 2.5 volumes of ethanol. A dilute 1.8%

(w/v) NaCl solution was used to suspend the white cells prior to lysis in each procedure. Both procedures used phenol and chloroform to deproteinize the cell digest.

5   Longmire, J. L. et al, "A Rapid and Simple Method for the Isolation of High Molecular Weight Cellular and Chromosome-Specific DNA in Solution Without the Use of Organic Solvents," Nucleic Acids Research, Vol. 15, no. 2, p. 859 (1987) describes a method of nontoxic DNA extraction after the digestion 10   of cell lysates with SDS and protease K. The SDS and digestion products are removed by dialysis with 4 x 60 minute washes using 20% polyethylene glycol (PEG). The sample volume decreased and the salts are removed by dialysis with 2 x 30 minute washes with 10 mM Tris-HCl 15   pH 8.0, 1 mM EDTA (TE buffer).

           Leadon, S. A. et al, "A Rapid and Mild Procedure for the Isolation of DNA from Mammalian Cells," Analytical Biochemistry, Vol. 20, pp. 282-288 (1982) describes a nontoxic method for the isolation of 20   DNA. Cells are placed on a polycarbonate filter. Cell lysis and digestion with SDS and protease K are carried out on the filter. The solution is allowed to flow through the filter by gravity. The DNA is then removed from the filters by treatment with DNase and 25   0.5 ml water. The DNA can also be removed from the filter by irradiation with 1000-1500 rad gamma rays followed by an aqueous wash.

           Smith, K.C. et al described the use of ammonium sulfate fractionation to separate amino-acid 30   specific RNA. They begin with transfer RNA extracted from guinea pig liver using a phenol procedure to eliminate protein.

           Hirt, B., J. Mol. Biol., Vol. 26, pp. 365-369 (1967) describes an extraction method for purifying low

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molecular weight nucleic acids such as DNA and RNA away
from high molecular weight DNA and proteins. The
procedure is performed by resuspending cells in a
buffered solution and lysing the cells by the addition
5   of SDS. Cell lysis can be performed at room
temperature or 37°C for 10 to 30 minutes. The buffered
solution usually contains 10 mM EDTA, pH 8.0 to chelate
bivalent cations. An alternative of the lysis
procedure resuspends cells directly in 1% SDS, 10 mM
10   EDTA pH 8.0. After cell lysis, NaCl is added to obtain
a 1 M final contration. The solution is cooled to 4°C
by incubation on ice for 30-60 minutes. At 4°C, high
molecular weight DNA, protein and SDS precipitates.
After incubation of 4°C, the solution is centrifuged at
15   4°C for 20-30 minutes at 10,000 rpm to pelletize the
precipitate. The supernatant fluid is then decanted.
The supernatant fluid contains the bulk of the low
molecular weight DNA and RNA from the cells. The
processing of the supernatant fluid at this point
20   depends on the desired use of the nucleic acid. Three
methods of processing the supernatant fluid that have
been used are: 1) addition of 2 to 2.5 volumes of
ethanol to the supernatant to precipitate the nucleic
acids and recovery of the precipitated nucleic acids by
25   centrifugation; 2) removal of the NaCl and residual SDS
by dialysis against a buffer; and 3) extraction of the
supernatant fluid using phenol and/or chloroform to
remove residual protein and SDS prior to ethanol
preciptation or dialysis.
30   Thus, the prior art has long sought a more
efficient, simple and less tedious method to remove
protein from a biological sample prior to isolation or
separation of nucleic acids. The time involved in the
phenol-chloroform separation procedure, the expense

related to waste disposal and the precautions required in handling these two chemicals does not make this procedure well-suited for large-scale mass extraction or for small laboratory use.

5 Summary of the Invention

An object of the present invention is to develop a fast and efficient method for isolating nucleic acids from a biological sample such as a cell digest or cell lysate.

10 A further object of the present invention is to provide a method for isolating nucleic acids which does not require the use of hazardous chemicals and maintains a high level of occupational safety for the technician.

15 An additional object of the present invention is to provide a method for precipitating protein present in a biological sample which uses economical and convenient materials.

The foregoing objectives have been realized

20 by a method for isolating nucleic acids from a biological sample which involves dissociating protein present in a biological sample containing nucleic acids; adding a sufficient amount of organic or inorganic salt to precipitate the protein; mixing the

25 biological sample containing the salt for a time sufficient to achieve a satisfactory dispersion of salt through the biological sample to obtain a protein precipitate; separating the precipitated protein from the biological sample; and finally separating the

30 nucleic acid remaining in the biological sample.

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Detailed Description of the Preferred Embodiments

The method of the present invention is suitable for isolating or separating nucleic acids from a biological sample. A biological sample for the purpose of the present invention is defined as any sample or composition which contains nucleic acids. Such samples may include a cell lysate, a cell digest, a sample containing cell leucocytes, a tissue sample, a urine sample, a blood stain or the like. Suitable samples may be obtained from the spleen, kidney, heart, muscle, spermatozoa, intestine, skin, blood and the like from a variety of living creatures whose cells contain nucleic acids including mammalian organisms such as humans. Suitable samples may also include microorganisms such as bacterial cells. Those skilled in the art would recognize suitable biological samples from which nucleic acids such as DNA and RNA may be extracted.

The term, biological sample, is preferably used when describing the present invention since numerous techniques in the area of biotechnology require the isolation of nucleic acids. Typical uses include restriction enzyme digestion, ligation, cloning and the like. The type of sample used for such nucleic acid isolations may vary.

Initially, the proteins including nucleoproteins present in the biological sample are dissociated. Sometimes the term denaturing is used to describe this step. Such techniques are well-known in the art. At the present time the use of proteinase K and SDS is preferred as noted in Molecular Cloning, supra. The product obtained is oftentimes referred to as a cell digest.

Any type of salt may be used in the practice
of the present invention. Obviously, the salt must be
selected so as not to interfere with the compounds used
in any of the other steps during the isolation of the
5   nucleic acids. Suitable salts include all organic
salts and all inorganic salts. A representative
listing of salts may be obtained by reference to any
standard chemistry text. One such text is Lange's
Handbook of Chemistry, thirteenth edition, editor John
10   A. Dean, 1985, which is incorporated herein by
reference in its entirety.

In its simplest terms a salt can be thought
of as an association between a cation and an anion.
Suggested anions in the practice of the present
15   invention include but are not limited to chloride,
bromide, fluoride, iodide, borate, hypobromite,
hypochlorite, nitrate, nitrite, hyponitrite, sulfate,
disulfate, sulfite, sulfonate, phosphate, diphosphate,
phosphite, phosphonate, diphosphonate, perchlorate,
20   perchlorite, oxalate, malonate, succinate, lactate,
carbonate, acetate, benzoate, citrate, tosylate,
permanganate, manganate, propanolate, propanoate,
ethandioate, butanoate, propoxide, chromate,
dichromate, selenate, orthosilicate, metasilicate,
25   pertechnetate, technetate, dimethanolate, dimethoxide,
thiocyanate, cyanate, isocyanate, 1,4-
cyclohexanedithiolate, oxidobutanoate,
3-sulfidocyclobutane-1-sulfonate, 2-(2-
carboxylatoethyl)-cyclohexanecarboxylate, 2-amino-4-
30   (methylthio)-butanoate and the like.

Suggested cations include but are not limited
to sodium, potassium, calcium, magnesium, ethylamine,
diethylamine, triethylamine, benzylamine,
dimethylaniline, N-methylmorpholine, amyloride,

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ammonium, lithium, berylium, cesium, zinc, chromium,
copper, aluminum, barium, strontium, iron, tin, nickel,
barium, silver, platinum, palladium, titanium and the
like.

5   The preferred salt depends on a variety of
factors, for instance, if ethanol is used in the final
extraction, the salt should preferably be soluble in
ethanol so that the residual salt does not precipitate
out along with the nucleic acids in the final
10   isolation. Sulfate salts are typically not soluble in
ethanol. It would also be advantageous for the salt to
be neutral so as not to significantly change the pH or
denature the DNA. Further, if SDS is used to digest
the protein, it would be preferable if the cation of
15   the salt did not react with anionic SDS which may be
present in the nucleic acid digest since it may result
in the formation of a sludge which could float on top
of the aqueous layer. Under certain conditions and in
certain concentrations, heavy metal salts may damage
20   nucleic acids by causing cleavage of phosphodiester
bonds. Thus, care must be taken when selecting a heavy
metal salt so as not to damage the DNA and RNA present
in the biological sample.

         As is obvious from the groups of suggested
25   cations and anions presented above, any inorganic or
organic salt is suitable to precipitate the protein in
the biological sample of the present invention.
Preferred inorganic salts include sodium chloride,
potassium chloride, magnesium chloride and calcium
30   chloride. Sodium chloride is more preferred. Of the
organic salts, ammonium acetate, sodium acetate,
potassium acetate and sodium benzoate are preferred.
Ammonium acetate is the most preferred salt among both
the inorganic and organic salts.

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It is believed that the salt dehydrates and
thus precipitates cellular proteins which may be
present in the biological sample. Thus, it is
desirable that the salt have a high solubility in water

5   to allow enough salt molecules to tie up water
molecules so that the protein readily dehydrates and
precipitates. The amount of salt necessary in the
practice of the present invention is an amount
sufficient to precipitate protein contained in the

10   biological sample. As noted above, a wide variety of
types of biological samples are suitable in the
practice of the present invention. And depending on
the origin of the sample varying amounts of protein and
nucleic acid may be present. Further, some of the

15   biological samples may require a substantial amount of
clean-up prior to isolation of the nucleic acids. The
amount of salt may range from about 0.01 weight % to
about 500 weight % based on the total weight of the
biological sample. Preferably, the range is from about

20   0.1 weight % to about 50 weight %. The salt is
preferably added in the form of a saturated solution,
however, the salt may be added in solid form or as a
dilute solution. For example, in the case of NaCl, a
saturated solution is about a 35% (w/v) solution.

25   After addition of the salt, the biological
sample must be mixed in order to obtain a desirable
dispersion of salt throughout the biological sample.
In most cases, shaking the biological sample containing
the salt for approximately fifteen seconds is

30   sufficient. However, depending on the size of the
sample, the type of sample, the salt selected, the
method of mixing, the temperature of the environment,
and like factors, the time for mixing can range
anywhere from approximately three seconds to

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approximately twenty minutes. The precise mixing time
is not critical but rather the biological sample
containing salt must be mixed for a sufficient time to
achieve a satisfactory dispersion of salt throughout
5   the biological sample and thereby obtain a protein
precipitate.

Following the mixing step, the precipitated
protein may be separated from the biological sample and
the nucleic acid-rich supernatant recovered by any
10   technique known in the art. One such technique is
centrifuging the biological sample and the protein
precipitate. A single 15 minute spin is generally
sufficient and the nucleic acid-rich supernatant may
then be readily separated from the precipitated
15   protein. Alternatively, the biological sample
containing the precipitated protein may simply be
filtered to obtain the nucleic acid-rich liquid.

The nucleic acids in the nucleic acid-rich
supernatant may then be separated from the biological
20   composition using any technique known in the art.
General methods for precipitating nucleic acid include
the use of alcohols, for example, ethanol, isopropanol
and the like, or alternatively, polymin-P,
streptomycin sulfate, a metal such as copper and the
25   like. This latter technique is particularly useful in
the case of high molecular weight nucleic acids.

The most widely used method for concentrating
nucleic acids is precipitation with ethanol. The
ethanol is preferably at ambient temperature, however,
30   a wide range of temperatures is suitable in the
practice of the present invention. The temperature
must be sufficient to allow the precipitation of
nucleic acid. Obviously, it is also desirable that any
other substances also present in the sample not

precipitate out along with the nucleic acid. The
precipitate in this widely used method is recovered by
centrifugation and redissolved in an appropriate buffer
at the desired concentration. This technique is rapid

5   and quantitative even with nanogram amounts of nucleic
acid. If the concentration of monovalent cations
present in the nucleic acid sample is too high it can
be diluted with TE
(pH 8.0). If the concentration of salts is too low, an

10   appropriate salt solution may be added. Suggested salt
solutions include sodium acetate, sodium chloride and
ammonium acetate.

Either DNA and/or RNA may be isolated from
the biological sample. RNA requires a slightly higher

15   concentration of ethanol to be precipitated from
solution than does DNA. RNA generally requires about
2.5 volumes of ethanol resulting in 71% ethanol final
concentration. However, obviously a variety of
techniques are known in the art to obtain DNA and/or

20   RNA. A detailed description of methods for the
concentration or precipitation of nucleic acids is set
forth in Maniatis et al, supra. The entire reference
by Maniatis et al is incorporated herein by reference
in its entirety.

25   The isolated DNA and/or RNA can then be used
in any standard molecular biological application,
including restriction enzyme digestion, ligation and
cloning. Additionally, samples can be further
processed using DNase and organic extraction to yield

30   RNA suitable for northern blot analysis.

In order to further illustrate the present
invention and the advantages thereof, the following
specific examples are given, it being understood that

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same are intended only as illustrative and in nowise
limiting.

EXAMPLE 1

SODIUM CHLORIDE

5   A tube of anticoagulated blood was
centrifuged for approximately 20 minutes at 2000 RPM to
separate the cells from the plasma. The white cell
(leucocyte) buffy coat was transferred to a 15 ml
polypropylene tube. Approximately 9 ml of red cell
10   lysis buffer (0.144 M NH_4Cl, 0.001 M $NaHCO_3$) was added
to the polypropylene tube to hemolyze red blood cells.
The polypropylene tube was inverted several times and
maintained at room temperature for approximately 20
minutes. The tube was spun for 15 minutes at 2000 RPM
15   to separate out the white blood cells. The supernatant
was discarded. A white cell pellet remained.

Approximately 3 ml nuclei lysis buffer (10 mM
Tris-HCl, 400 mM NaCl and 2 mM Na_2EDTA, pH 8.2) was
added to resuspend the white cell pellet.
20   Approximately 0.2 ml 10% sodium dodecyl sulfate (SDS)
and 0.5 ml protease K solution (1 mg protease K in 1%
sodium dodecyl sulfate (SDS) and 2 mM Na_2EDTA) was
added to the white cell suspension to irreversibly
inactivate the nucleases and to dissociate the
25   nucleoproteins. The suspension was inverted several
times and maintained at 37°C for a minimum of three
hours.

Approximately 1 ml of saturated NaCl solution
was added to the nucleic acid digest for precipitation
30   and removal of the protein present in the nucleic acid
digest. The mixture was shaken vigorously for 15
seconds and centrifuged at 2500 RPM for 15 minutes.
The DNA rich supernatant was transferred to a 15 ml

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polypropylene tube. Approximately 2 volumes of room temperature ethanol was added to the polypropylene tube to precipitate DNA. This amount of ethanol is insufficient to precipitate the RNA therefore the RNA

5   stayed in solution. The tube was inverted several times until the DNA strands formed and began to stick together. The DNA strands appeared fluffy and white. The DNA was transferred using a plastic spatula to a 1.5 ml microcentrifuge tube containing approximately

10   100 to 200 microliters of 10 mM Tris-HCl and 0.2 mM Na$_2$EDTA, pH 7.5 to dissolve the DNA. The DNA was then diluted 1 to 100 in deionized water and quantitated at 260/280 nm (DNA quantity/residual protein quantitation) on a spectrophotometer. Five separate runs were

15   conducted using NaCl and the following 260/280 values were obtained: 1.8, 1.77, 1.9, 1.76 and 1.78. A value of 1.5 or greater is considered desirable.

EXAMPLE 2

POTASSIUM CHLORIDE

20   Example 2 was conducted in a similar manner as Example 1 except that approximately 1.0 ml of a saturated solution of KCl was added to the cell digest. Fluffy, white strands of DNA were obtained.

EXAMPLE 3

MAGNESIUM CHLORIDE

25   Example 3 was conducted in a similar manner as Example 1 except that approximately 1.0 ml of a saturated solution of MgCl$_2$ was added to the cell digest. Fluffy, white strands of DNA were obtained.

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EXAMPLE 4
SODIUM ACETATE

Example 4 was conducted in a similar manner as Example 1 except that approximately 1.0 ml of a saturated solution of sodium acetate was added to the cell digest. Six separate runs were conducted using sodium acetate and the following 260/280 values were obtained: 1.76, 1.83, 1.8, 1.82, 1.84 and 1.9.

EXAMPLE 5
AMMONIUM ACETATE

Example 5 was conducted in a similar manner as Example 1 except that approximately 1.0 ml of a saturated solution of ammonium acetate was added to the cell digest. The white blood cell sample provided the following 260/280 values: 1.87, 1.84, 1.86, 1.87, 1.87, 1.88, 1.87, 1.86, 1.87, 1.87, 1.88, 1.87, 1.86, 1.84, 1.88 and 1.87.

EXAMPLE 6
AMMONIUM ACETATE

Example 6 was conducted in a similar manner as Example 5 using ammonium acetate, however, the biological sample for Example 6 was derived from tissue. A 260/280 value of 1.77 was obtained.

EXAMPLE 7
RFLP STUDY USING DNA SAMPLE

Example 7 was conducted in a similar manner as Example 1 except that DNA was extracted from anticoagulated blood from five separate individuals. After extraction the samples were quantitated and five ug of each sample was restricted with 8 U/ug of Apa I. DNA was incubated according to manufacturer

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specifications (New England Biolabs, Inc.). DNA was
electrophoresed in agarose gels, stained with ethidium
bromide to check for completeness of restriction and
Southern blotted. Membranes were hybridized with human
5   DNA probes specific for detecting Apa I restriction
fragment length polymorphisms. Bands were detected
with the non-isotopic detection system as described by
Dykes et al, "The Use of Biotinylated DNA Probes for
Detecting Single Copy Human Restriction Fragment Length
10   Polymorphisms Separated by Electrophoresis",
Electrophoresis, Vol. 7, pp. 278-282 (1986). Observed
patterns demonstrated no partial restrictions, thus
demonstrating that the salting-out extraction
procedure did not inhibit the restriction enzymes.

15   EXAMPLE 8
                    RFLP STUDY USING DNA SAMPLE
             Example 8 was conducted in the same manner as
     Example 7 except that extracted DNA samples were
     restricted separately with 6 U/ug of Tag I and 9 U/ug
20   of Msp I. Observed bands after hybridization and non-
     isotopic detection demonstrated complete restriction.

                            EXAMPLE 9
                    RFLP STUDY USING DNA SAMPLE
             Example 9 was conducted in the same manner as
25   Example 7
     except that extracted DNA samples were restricted with
     10 U/ug of Rsa I. Observed bands after hybridization
     and non-isotopic detection demonstrated complete
     restriction.

30   While the invention has been described in
     terms of various preferred embodiments, those skilled

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in the art will appreciate that various modifications,
substitutions, omissions, and changes may be made
without departing from the spirit thereof.
Accordingly, it is intended that the scope of the
5   present invention be limited solely by the scope of the
following claims.

WHAT IS CLAIMED IS:

1   1.   A method for isolating nucleic acids
2   from a biological sample, said method comprising:
3   a)   dissociating protein present in a
4   biological sample containing nucleic acids;
5   b)   adding a sufficient amount of
6   organic or inorganic salt to precipitate said protein;
7   c)   mixing said biological sample
8   containing said salt for a time sufficient to achieve a
9   satisfactory dispersion of said salt throughout said
10 biological sample and to obtain a protein precipitate;
11   d)   separating said precipitated protein
12 from said biological sample; and
13   e)   separating said nucleic acid remaining in
14 said biological sample.

1   2.   The method as claimed in claim 1 wherein
2   said protein is dissociated in step a) using a
3   proteolytic enzyme or an ionic detergent.

1   3.   The method as claimed in claim 2 wherein
2   said protein is dissociated in step a) using proteinase
3   K and sodium dodecyl sulfate.

1   4.   The method as claimed in claim 1 wherein
2   said salt is sodium chloride, potassium chloride,
3   magnesium chloride, calcium chloride, ammonium acetate,
4   sodium acetate, potassium acetate or sodium benzoate.

1   5.   The method as claimed in claim 4 wherein
2   said salt is ammonium acetate.

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1   6.   The method as claimed in claim 1 wherein
2   said salt is present in the range of about 0.01 wt % to
3   about 500 wt % based upon the total weight of the
4   biological sample.

1   7.   The method as claimed in claim 1 wherein
2   said precipitated protein is separated by centrifuging.

1   8.   The method as claimed in claim 1 wherein
2   said precipitated protein is separated by filtration.

1   9.   The method as claimed in claim 1 wherein
2   the nucleic acid is separated in step e) by the
3   addition of a sufficient amount of alcohol to obtain a
4   nucleic acid precipitate.

1   10.   The method as claimed in claim 9
2   wherein a sufficient amount of alcohol is added to
3   precipitate primarily DNA.

1   11.   The method as claimed in claim 9 wherein
2   a sufficient amount of alcohol is added to precipitate
3   primarily RNA.

1   12.   A method for isolating DNA from a
2   biological sample, said method comprising:
3   a)   dissociating protein present in a
4   biological sample containing DNA;
5   b)   adding a sufficient amount of a salt
6   selected from the group consisting of sodium chloride,
7   potassium chloride, magnesium chloride, calcium
8   chloride, ammonium acetate, sodium acetate, potassium
9   acetate and sodium benzoate, to precipitate said
10 protein;

11   c)   mixing said biological sample containing
12   said salt for a time sufficient to achieve a
13   satisfactory dispersion of said salt through said
14   biological sample to obtain a protein precipitate;
15   d)   separating said precipitated protein
16   from said biological sample; and
17   e)   separating said DNA remaining in said
18   biological sample.

1   13.   The method as claimed in claim 12
2   wherein said protein is dissociated in step a) using
3   proteinase K and sodium dodecyl sulfate.

1   14.   The method as claimed in claim 12 wherein said
2   salt is ammonium acetate.

1   15.   The method as claimed in claim 12 wherein the
2   DNA is separated in step e) by the addition of a
3   sufficient amount of alcohol to precipitate DNA.

1   16.   The method as claimed in claim 15 wherein the
2   alcohol is ethanol.

1   17.   The method as claimed in claim 16 wherein the
2   ethanol is added at ambient temperature.

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4) C07H 15/12; C12P 19/34; C07G 17/00; C07K 1/14, 3/12
U.S. Cl.: 530/344,412 435/91, 267 536/27

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
U.S.	530/344, 412 435/91, 267 536/27

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched 8

Chemical Abstracts Data Base (CAS) 1967-1989 Keywords: isolation
purification/preparation/nucleic acids/RNA/DNA/without or absence
phenol or organic solvents/proteins/protein/precipitate

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X — Y	Clin. Chem. Vol. 31: 1985, January, Buffone, G.J. "Isolation of DNA from Biological Specemins without Extraction with Phenol". pp. 164-165	1-4,6,7, 9,10,12,13 15-17 — 5,8,11,14
X — Y	Methods in Enzymology, Vol. 65, 1980, April, Davis, R.W., "Rapid DNA Isolations for Enzymatic and Hybridization Analysis". pp. 404-411.	1-4,6,7, 9,10,12,13, 15-17 — 5,8,11,14
Y	US, A, 4,427,580 (Cornell Research Foundation) 24 January, 1984 See p. 4, line 54 and Figure 1.	8
Y	Scopes, R. Protein Purification 1982 Springer Verlag New York pp. 47-48.	5,14
Y	Maniatis, T. Molecular Cloning, 1982 Cold Spring Harbor, New York, p. 401, 461-462.	11

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
31 March 1989	0 4 MAY 1989
International Searching Authority	Signature of Authorized Officer
ISA/US	ANNE BROWN

Form PCT/ISA/210 (second sheet) (Rev.11-87)